Activities of Fosfomycin, Tigecycline, Colistin, and Gentamicin against Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* in a Foreign-Body Infection Model

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Limited antimicrobial agents are available for the treatment of implant-associated infections caused by fluoroquinolone-resistant Gram-negative bacilli. We compared the activities of fosfomycin, tigecycline, colistin, and gentamicin (alone and in combination) against a CTX-M15-producing strain of *Escherichia coli* (Bj HDE-1) *in vitro* and in a foreign-body infection model. The MIC and the minimal bactericidal concentration in logarithmic phase (MBC_{log}) and stationary phase (MBC_{stat}) were 0.12, 0.12, and 8 μg/ml for fosfomycin, 0.25, 32, and 32 μg/ml for tigecycline, 0.25, 0.5, and 2 μg/ml for colistin, and 2, 8, and 16 μg/ml for gentamicin, respectively. In time-kill studies, colistin showed concentration-dependent activity, but regrowth occurred after 24 h. Fosfomycin demonstrated rapid bactericidal activity at the MIC, and no regrowth occurred. Synergistic activity between fosfomycin and colistin *in vitro* was observed, with no detectable bacterial counts after 6 h. In animal studies, fosfomycin reduced planktonic counts by 4 log_{10} CFU/ml, whereas in combination with colistin, tigecycline, or gentamicin, it reduced counts by >6 log_{10} CFU/ml. Fosfomycin was the only single agent which was able to eradicate *E. coli* biofilms (cure rate, 17% of implanted, infected cages). In combination, colistin plus tigecycline (50%) and fosfomycin plus gentamicin (42%) cured significantly more infected cages than colistin plus gentamicin (33%) or fosfomycin plus tigecycline (25%) ($P < 0.05$). The combination of fosfomycin plus colistin showed the highest cure rate (67%), which was significantly better than that of fosfomycin alone ($P < 0.05$). In conclusion, the combination of fosfomycin plus colistin is a promising treatment option for implant-associated infections caused by fluoroquinolone-resistant Gram-negative bacilli.

Prosthetic joint infection (PJI) is a rare but difficult-to-treat complication which requires combined surgical and antimicrobial treatment and is associated with significant morbidity and health care costs (1–3). Staphylococci cause more than half of all PJIs (4), but Gram-negative rods are implicated in 6 to 23% (5–7). While rifampin-containing regimens showed high cure rates in staphylococcal PJIs (2), fluoroquinolones demonstrated good activity against PJI caused by Gram-negative rods (8). Besides a favorable pharmacokinetic profile, fluoroquinolones have excellent activity against Gram-negative biofilms (9, 10).

Escherichia coli is one of the predominant organisms among Gram-negative bacilli causing PJI (6, 7, 11, 12). In this organism, the emergence of extended-spectrum-β-lactamase (ESBL)-producing strains harboring the plasmid-acquired gene for CTX-M15 β-lactamase is of particular concern (13, 14). In ESBL-producing strains, the resistance to beta-lactams (except carbapenems) is frequently associated with resistance to other antibiotics, including fluoroquinolones, aminoglycosides, and cotrimoxazole. In the absence of new antimicrobial agents (15), "old" antibiotics have been reconsidered and evaluated in multidrug-resistant Gram-negative bacilli, including fosfomycin and colistin (16–18).

Fosfomycin has a broad activity against Gram-positive and Gram-negative bacteria and has mainly been used for treatment of uncomplicated urinary tract infections, with good efficacy and tolerability (19, 20). High concentrations of fosfomycin were reported in bone in patients with diabetic foot osteomyelitis, likely due to its structural similarity to hydroxyapatite (21). For treatment of endocarditis, fosfomycin showed synergistic activity with

high-dose daptomycin against *Staphylococcus aureus* (22). Tigecycline, the first glycylcycline in clinical use, has an expanded broad-spectrum activity, including activity against resistant Gram-negative bacilli, when combined with other antimicrobials (23). Colistin has been increasingly used in the last decade because of the emergence of multidrug-resistant Gram-negative bacilli in the past years, especially in combination with other drugs (17, 24).

In this study, we investigated the activity of fosfomycin, tigecycline, colistin, and gentamicin, alone and in combination, against a well-characterized ESBL-producing clinical strain of *E. coli in vitro* and in a foreign-body infection model (25–28). This animal model has been previously used for studying the activity of antimicrobial combinations against implant-associated infections (26, 29).

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MATERIALS AND METHODS

Test strain. *E. coli* Bj HDE-1 [13], an ESBL-producing clinical strain that is resistant to ciprofloxacin, was used for *in vitro* and animal experiments. As control strain for susceptibility testing, the ciprofloxacin-susceptible *E. coli* strain ATCC 25922 was used. Strains were stored at −70°C by using a cryovial bead preservation system (Microbank; Pro-Lab Diagnostics, Richmond Hill, Ontario, Canada). One cryovial bead was cultured overnight on Columbia sheep blood agar plates (Becton, Dickinson, Heidelberg, Germany). Inocula were prepared from subcultures of one colony, resuspended in 5 ml of Trypticase soy broth (TSB), and incubated overnight at 37°C without shaking.

Antimicrobial agents. Fosfomycin was provided as purified powder by the manufacturer (InfectoPharm, Heppenheim, Germany), tigecycline by Pfizer (Pfizer, Zurich, Switzerland), colistin by Sanofi-Aventis (Paris, France), and gentamicin by Essex Chemie (Lucerne, Switzerland). Stock solutions of appropriate concentrations were prepared in sterile pyrogen-free water.

Antimicrobial susceptibility. The susceptibility of *E. coli* to fosfomycin, tigecycline, colistin, and gentamicin was determined in triplicate by using a standard inoculum of 5×10^5 CFU/ml adjusted from overnight cultures. The MIC was determined in Mueller-Hinton broth (MHB) by the macrodilution method, according to Clinical and Laboratory Standards Institute (CLSI) guidelines [30]. In brief, 10 2-fold serial dilutions of each antimicrobial agent were prepared in 2 ml MHB in plastic tubes. Tubes were inoculated below the meniscus and incubated for 18 h at 37°C without shaking. After the determination of MIC (defined as the lowest antimicrobial concentration inhibiting visible growth), tubes without visible growth were vigorously vortexed and incubated for an additional 4 h at 37°C without shaking. Aliquots of 100 μl were plated on blood agar plates, and the number of viable bacteria was determined. The minimum bactericidal concentration during logarithmic growth (MBC_{log}) was defined as the lowest antimicrobial concentration which killed ≥99.9% of the initial bacterial count (i.e., ≥3 $\log_{10}$ CFU/ml) [31]. The minimum bactericidal concentration during stationary growth phase (MBC_{stat}) was determined in nutrient-restricted medium consisting of 0.01 M phosphate-buffered saline (pH 7.4), as previously described [27]. In this medium, counts of *E. coli* remained within ±10% of the initial inoculum for >36 h.

Time-kill studies. The activities of fosfomycin, tigecycline, colistin, and gentamicin (alone and in combination) were evaluated by time-kill studies using an inoculum of ~1×10^6 CFU/ml of *E. coli*. In time-kill studies, a higher inoculum was used than in antimicrobial susceptibility tests in order to investigate the synergistic and antagonistic effects of drug combinations. Antibiotic dilutions were prepared in 10 ml of MHB adjusted to final concentrations of 0.5, 1, and 4× the MIC of the test strain. MHB without antibiotics served as a growth control; MHB without *E. coli* served as a negative control. The CFU were enumerated after 0, 2, 4, 6, and 24 h of incubation at 37°C by plating aliquots of appropriate dilutions on Mueller-Hinton agar (MHA). Antibiotic dilutions of ≥10-fold were used to assess numbers in the range of 10 to 250 CFU per plate and to minimize the antimicrobial carryover effect. The quantification limit was set at 200 CFU/ml (i.e., 10 CFU in 50 μl at a 10-fold dilution). Killing was expressed as reduction in $\log_{10}$ CFU/ml (mean ± standard deviation [SD]). Synergism was defined as a >2-$\log_{10}$ increase in killing with the drug combination at 24 h compared to killing achieved with the more active single drug. Antagonism was defined as a >2-$\log_{10}$ decrease in killing with the drug combination at 24 h compared to killing achieved with the more active single drug.

Emergence of antimicrobial resistance *in vitro*. The *in vitro* emergence of resistance was screened by plating *E. coli* on MHA containing fosfomycin, tigecycline, or colistin at 4× the MIC. For this purpose, several colonies of *E. coli* from the agar plate were suspended in normal saline, adjusted to a turbidity of McFarland 0.5 (≈1×10^8 CFU/ml), plated in 0.1-ml aliquots on MHA, and incubated for 48 h. The antimicrobial sus-

ceptibility of colonies growing on MHA was determined by Etest (AB Biodisk, Solna, Sweden).

Evaluation of antibiofilm activity using microcalorimetry. Biofilm was formed on porous sintered glass beads (diameter, 4 mm; pore size, 60 μm; surface area, approximately 60 cm²). Glass beads were placed in Luria broth (LB), inoculated with 2 colonies of *E. coli*, and incubated at 37°C. After 3 h, 12 h, and 24 h, beads were removed from the LB, washed three times with normal saline, and incubated in 2 ml of LB containing serial dilutions of fosfomycin, tigecycline, colistin, and gentamicin, alone and in combination, at concentrations of 0.12 to 256 μg/ml. After another 24 h, beads were washed three times with normal saline and placed in microcalorimetry ampoules containing 3 ml LB to detect heat production of residual bacteria. Measurements were performed in an isothermal 48-channel batch calorimeter (thermal activity monitor, model 3102 TAM III; TA Instruments, New Castle, DE), calibrated at 37°C ± 0.00001°C with a lower limit of heat-flow detection of 0.25 μW. All experiments were performed in triplicate. The minimal heat inhibition concentration (MHIC) was defined as the lowest antimicrobial concentration inhibiting heat production with a delay of ≥24 h, as previously described [25, 32].

Animal model. An established foreign-body infection model in guinea pigs was used, as described previously [25, 28]. Male albino guinea pigs (Charles River, Sulzfeld, Germany) were kept at the animal facility of our institution. Animal experiments were performed according to the Swiss veterinary law regulations. The study protocol was approved by the Institutional Animal Care and Use Committee. In this model, four sterile polytetrafluoroethylene (Teflon) cages (32 mm by 10 mm) perforated with 130 regularly spaced holes 1 mm in diameter (Angst-Pfister, Zurich, Switzerland) were subcutaneously implanted under aseptic conditions in the flanks of guinea pigs weighing at least 450 g. Animals were anesthetized with a subcutaneous injection of ketamine (20 mg/kg) and xylazine (4 mg/kg). Two weeks after surgery and upon complete healing of the surgical wounds, sterility of the cages was verified by culture of the aspirated cage fluid. Contaminated cages were excluded from further studies. For the treatment studies, cages were infected by percutaneous inoculation of 200 μl containing 7×10^4 CFU of *E. coli* (i.e., day 0). The establishment of infection was confirmed 24 h after infection by quantitative culture of aspirated cage fluid (i.e., day 1).

Antimicrobial treatment studies. Antimicrobial treatment was initiated 24 h after infection (day 1). Three animals (each holding four cages) were randomized into each of the following treatment groups (drugs were administered alone or in combination): untreated control (sterile saline); fosfomycin (150 mg/kg), tigecycline (10 mg/kg), colistin (15 mg/kg), and gentamicin (10 mg/kg). All antimicrobial agents were administered every 12 h intraperitoneally for 4 days, i.e., a total of eight doses, as previously reported [28]. The dosing regimens for all tested antimicrobials were chosen according to pharmacokinetic studies performed in mice, rats, and rabbits, mimicking concentrations achievable in humans [33–40].

Activity against planktonic bacteria. Planktonic bacteria in the aspirated cage fluid were enumerated on day 4, just before administration of the last antimicrobial dose, and on day 10, which is 5 days after end of antimicrobial treatment. Bacterial counts were expressed as means ± SD of $\log_{10}$ CFU/ml. The quantification limit of planktonic bacteria was set at 1,000 CFU/ml (i.e., 10 CFU in 50 μl at a 10-fold dilution). Thus, for statistical analysis, a value of 3 $\log_{10}$ CFU/ml was assigned to negative cage fluid cultures. The treatment efficacy against planktonic bacteria was expressed as the reduction of CFU $\log_{10}$/ml in the cage fluid.

Activity against biofilm bacteria. Animals were sacrificed on day 10, and cages were removed under aseptic conditions. Each cage was separately placed in 5 ml TSB, vortexed for 30 s, and incubated at 37°C. After 24 h, cages were vortexed for 30 s and sonicated at 40 kHz for 1 min (BactoSonic, Bandelin, Berlin, Germany) to remove potential biofilm bacteria, as previously described [41]. Aliquots of 100 μl were plated on MHA (Becton, Dickinson) and assessed for bacterial growth 24 to 48 h later. The activity against biofilm bacteria was expressed as the cure rate,

TABLE 1 Susceptibility of *E. coli* Bj HDE-1 to fosfomycin, tigecycline, colistin, and gentamicin

Antimicrobial agent	MIC (μg/ml)	MBC_{log} (μg/ml)	MBC_{stat} (μg/ml)	MBC_{stat}/MBC_{log} ratio
Fosfomycin	0.12	0.12	8	66
Tigecycline	0.25	32	32	1
Colistin	0.25	0.5	2	4
Gentamicin	2	8	16	2

FIG 2 Time-kill curves with 0.5× the MIC of tigecycline (TIG), colistin (COL), fosfomycin (FOS), and gentamicin (GEN) in combinations against *E. coli* in log growth phase (inoculum, 10^6 CFU/ml). Values are means ± SD. The experiments were performed in triplicate. GC, growth control.
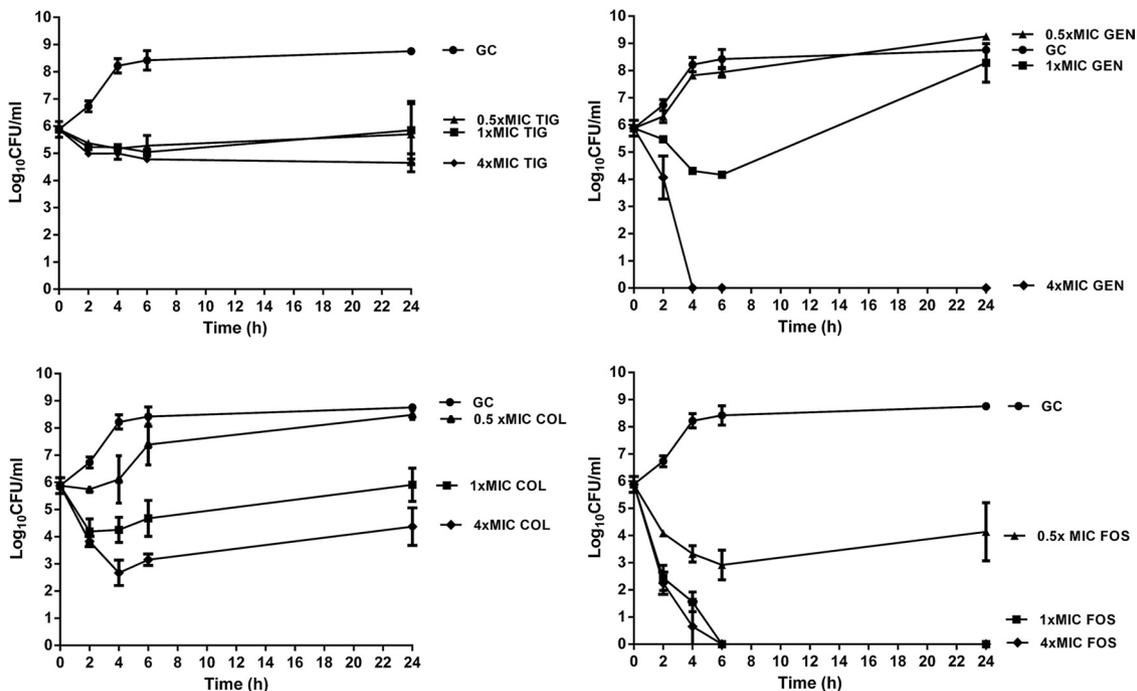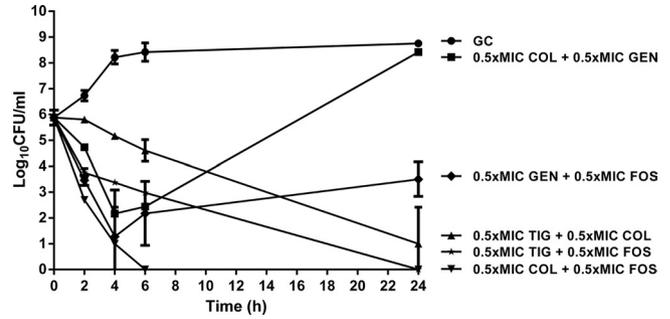
defined as the number of cage cultures without *E. coli* growth divided by the total number of cages in the treatment group.

Emergence of antimicrobial resistance *in vivo*. *E. coli* grown from explanted cages in TSB (i.e., treatment failures) was screened for emergence of resistance to fosfomycin, tigecycline, or colistin, as described for the emergence of *in vitro* resistance.

Statistical analysis. Comparisons were performed by using the Mann-Whitney U test for continuous variables and a two-sided χ^2 or Fisher's exact test for categorical variables, as appropriate. For all tests, differences were considered significant when *P* values were <0.05. Figures were plotted with GraphPad Prism (version 6.01) software (GraphPad Software, La Jolla, CA).

RESULTS

Antimicrobial susceptibility. Table 1 summarizes the antimicrobial susceptibility of *E. coli* to fosfomycin, tigecycline, colistin, and gentamicin. In logarithmic growth, fosfomycin and colistin killed *E. coli* at concentrations of <0.5 μg/ml, whereas gentamicin and tigecycline required higher concentrations (MBC_{log} of 8 μg/ml and 32 μg/ml, respectively). In stationary growth, the MBC_{stat} for colistin was low (2 μg/ml), that for fosfomycin was intermediate (8 μg/ml), and those for gentamicin and tigecycline were high (16 and 32 μg/ml, respectively).

Time-kill studies. Figure 1 shows the time-kill curves. Bacterial counts increased to 5×10^8 CFU/ml after 24 h without antimicrobials (growth control). With tigecycline, bacterial counts remained unchanged at 0.5× and 1× the MIC and decreased ~1 $\log_{10}$ CFU/ml at 4× the MIC at 24 h. With gentamicin, regrowth occurred at 0.5× and 1× the MIC at 24 h, but a rapid bactericidal activity with undetectable bacterial counts was observed at 4× the MIC after 4 h. Colistin showed concentration-dependent activity, with a maximum decrease of 3.1 $\log_{10}$ CFU/ml, but regrowth was observed after 24 h. Fosfomycin demonstrated rapid bactericidal activity, independent of the drug concentration at or above the MIC.

Figure 2 shows time-kill curves of antimicrobial combinations at fixed concentration of 0.5× the MIC. Tigecycline in combination with colistin and fosfomycin decreased bacterial counts at 24 h by 4.5 and 5.5 $\log_{10}$ CFU/ml, respectively. Regrowth was observed with gentamicin either in combination with colistin (to 3×10^8 CFU/ml) or

FIG 1 Time-kill curves with 0.5, 1, and 4× the MIC of tigecycline (TIG), colistin (COL), fosfomycin (FOS), and gentamicin (GEN) against *E. coli* in log growth phase (inoculum, 10^6 CFU/ml). Values are means ± SD. The experiments were performed in triplicate. GC, growth control.

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TABLE 2 Activity against *E. coli* Bj HDE-1 biofilms on glass beads, evaluated by microcalorimetry

Antimicrobial agent	MHIC (µg/ml)		
	3-h biofilm	12-h biofilm	24-h biofilm
Fosfomycin	0.12	<0.12	<0.12
Tigecycline	128	128	128
Colistin	32	32	32
Gentamicin	8	16	16

fosfomycin (to 3.8×10^3 CFU/ml) at 24 h. Synergistic activity was observed between fosfomycin and colistin with undetectable bacterial counts after 6 h and without regrowth at 24 h.

Emergence of antimicrobial resistance *in vitro*. No growth of *E. coli* was observed on MHA containing fosfomycin, colistin, or tigecycline at 4× the MIC.

Evaluation of antibiofilm activity using microcalorimetry. Table 2 summarizes the MHIC values for the antimicrobial agents tested. Fosfomycin showed excellent activity against biofilm on glass beads and suppressed heat production at or below the MIC (42). Tigecycline and colistin suppressed heat production only at high concentrations (128 and 32 µg/ml), whereas gentamicin showed intermediate activity against *E. coli* biofilm (at 2× the MBC_{log}, i.e., 16 µg/ml). No differences in antibiofilm activity were observed among 3-h, 12-h, and 24-h biofilms.

Antimicrobial treatment study. All cage fluid samples aspirated before infection were sterile. Twenty-four hours after infection, the mean ($\pm$ SD) concentration of bacteria in the cage fluid was 5.3 log_{10} CFU/ml ($\pm$ 0.5 log_{10} CFU/ml). Neither clearance of planktonic bacteria nor spontaneous cure of biofilm bacteria occurred in any cage from untreated animals.

(i) Activity against planktonic bacteria. Figure 3 shows the counts of planktonic bacteria in cage fluid during treatment and 5 days after end of treatment. In untreated animals, bacterial counts in cage fluid increased to 7.5 and 7.2 log_{10} CFU/ml, respectively, which corresponds to an increase of 2.2 and 1.9 log_{10} CFU/ml, respectively. During treatment, the count of planktonic bacteria (in log_{10} CFU/ml) decreased in comparison with untreated con-

trol by 2.1 (with gentamicin), 3.3 (with fosfomycin), 4.2 (with colistin), and 4.5 (with tigecycline). After treatment, regrowth was observed in animals treated with colistin and tigecycline (an increase compared to the bacterial counts during treatment of 1.3 and 1.6 log_{10} CFU/ml, respectively), whereas bacterial counts remained unchanged with gentamicin. With fosfomycin a decrease of 4.7 log_{10} CFU/ml was observed. After the end of treatment, all regimens showed significantly lower bacterial counts than untreated controls ($P < 0.01$, except for colistin, where $P < 0.05$).

When colistin or fosfomycin was added to gentamicin or tigecycline, bacterial counts were reduced by >3.0 log_{10} CFU/ml (during and after treatment), which was significantly lower than with gentamicin or tigecycline alone ($P < 0.01$). With the combination tigecycline plus colistin bacterial counts remained unchanged. All other combinations decreased bacterial counts (in log_{10} CFU/ml) by 1.5 (fosfomycin plus tigecycline), 1.6 (fosfomycin plus gentamicin), 1.2 (fosfomycin plus colistin) and 1.3 (colistin plus gentamicin), although they did not reach a 2-log_{10} CFU/ml reduction and were thus not synergistic. The combination of fosfomycin plus colistin was significantly more active against planktonic bacteria than each single drug ($P < 0.01$).

(ii) Activity against biofilm bacteria. Figure 4 shows the cure rates of all treatment regimens. Fosfomycin was the only single agent which was able to eradicate some *E. coli* biofilms (cure in 17% of cages). In combination, colistin plus tigecycline (50%) and fosfomycin plus gentamicin (42%) cured significantly more infected cages than colistin plus gentamicin (33%) or fosfomycin plus tigecycline (25%) ($P < 0.05$). The combination of fosfomycin plus colistin showed the highest cure rate (67%), which was significantly better than that of fosfomycin alone ($P < 0.05$).

Emergence of antimicrobial resistance *in vivo*. No resistant strains were detected in positive cultures from cages.

DISCUSSION

We investigated the activities of fosfomycin, tigecycline, colistin, and gentamicin against an ESBL-producing *E. coli* strain. This organism was chosen because it has been well characterized at the molecular level (13) and is resistant to ciprofloxacin, reflecting the
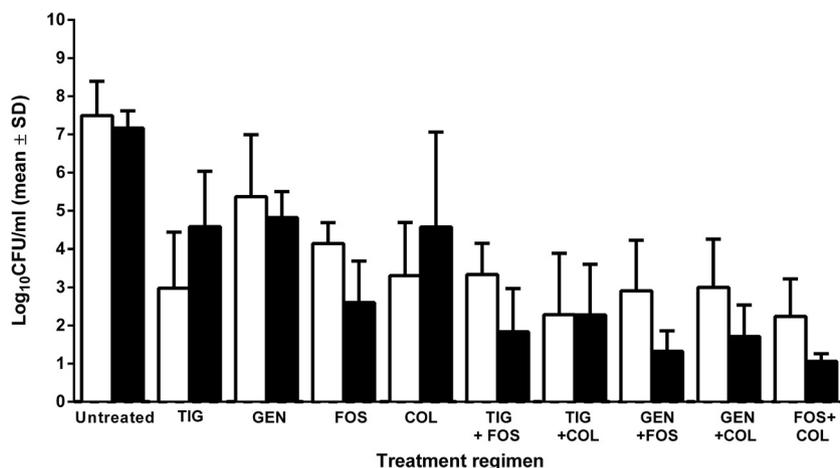

FIG 3 Activities against planktonic bacteria in cage fluid aspirated during treatment (i.e., day 5; open bars) and 5 days after end of treatment (i.e., day 10; closed bars). In each group, fluid from 12 cages (from 3 animals) was investigated. The *y* axis shows log_{10} CFU/ml in aspirated cage fluid, expressed as means $\pm$ standard deviations (SD).

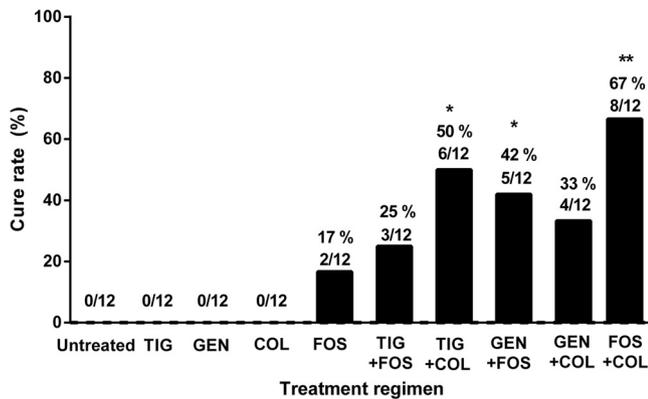Antimicrobial Agents and Chemotherapy

FIG 4 Rate of cure of cage-associated infection. The values are numbers of cage cultures without growth of *E. coli* divided by the total number of cages in the treatment group (*n* = 12). Significant differences compared to untreated controls are indicated with asterisks (*, $P < 0.05$; **, $P < 0.01$).

current epidemiology in PJI (7) and classifying this organism among difficult-to-treat organisms (4, 43, 44).

The test strain was highly susceptible to fosfomycin, tigecycline, and colistin (all MICs ≤ 0.25 μg/ml), as well as to gentamicin (MIC = 2 μg/ml), which is still in the susceptible range according to the EUCAST and CLSI guidelines. However, a reduction of ≥99.9% CFU/ml was achieved only with fosfomycin and colistin, both in logarithmic and stationary growth (at concentrations which are achievable in the clinical setting). This is in agreement with the reported bactericidal activity of fosfomycin and colistin against Gram-negative bacilli (45, 46). For gentamicin, the MBC_{log} was 8 and MBC_{stat} was 16 μg/ml, which are achievable with once-daily dosing in humans. In contrast, tigecycline exhibits bacteriostatic activity and does not seem to be effective as a single agent against implant-associated infections due to Gram-negative bacilli (23). However, synergy with colistin has been reported against MBL (VIM-1)- and ESBL (SHV-12)-producing *Klebsiella pneumonia* (47).

In the time-kill studies, bacterial regrowth was detected after 24 h in all experiments using colistin alone. Regrowth was previously reported with *Pseudomonas aeruginosa* (48) and *Acinetobacter baumannii* (49), which may possibly be explained by the short half-life of colistin (50). In contrast, colistin in combination with tigecycline or fosfomycin (but not with gentamicin) prevented regrowth at subinhibitory concentration (0.5× the MIC). Neither fosfomycin nor colistin prevented regrowth when combined with gentamicin. In a time-kill curve assay, the most active single agent was fosfomycin, which exhibited a synergistic effect in combination with colistin. Tigecycline combined with fosfomycin or colistin was also active against planktonic bacteria.

The microcalorimetry glass bead method mimics the Calgary biofilm device, where antibiotic activity against biofilms is determined by the antibiotic challenge and biofilm recovery method (7). Fosfomycin revealed the lowest MIHC, supporting other studies demonstrating its ability to kill bacteria in biofilms (20, 51). Interestingly, no correlation between the biofilm age and the antibiofilm activity was observed in our study.

In an animal study using the same animal model, *E. coli* counts increased in the cage fluid over 10 days, and no spontaneous cure occurred in untreated animals (52); the infection profile was also

similar to the one previously reported in the same experimental model (53).

The bacterial count of planktonic bacteria in cage fluid slightly decreased after the end of treatment in untreated control animals, but no spontaneous cure was observed. Reduction of bacterial counts can be explained due to an unfavorable environment (e.g., anoxic conditions), the biofilm mode of growth, or the postantibiotic effect. The presence of neutrophils may also prolong the *in vivo* postantibiotic effect (54).

Fosfomycin was the only single agent which achieved eradication of *E. coli* from cages, although the cure rate was only 17%. When fosfomycin was combined with colistin, the activity against both planktonic and biofilm bacteria was significantly improved, achieving a cure rate of 67%. This observation suggests that fosfomycin has potential for the treatment of Gram-negative implant-related infections (55), especially in combination with colistin (with or without tigecycline) in the case of fluoroquinolone resistance (7). Recently, fosfomycin showed improved activity against *P. aeruginosa* biofilms in a rat model of urinary tract infection (56). The small size of the fosfomycin molecule may explain its ability to penetrate established biofilm, including bacteria in stationary growth phase (57). Despite the fact that no emergence of resistance to fosfomycin, tigecycline, or colistin was observed in our study *in vitro* and *in vivo*, selection and/or emergence of resistant strains may occur during treatment. The emergence of colistin-heteroresistant subpopulations was recently reported (58).

In conclusion, the combination of fosfomycin and colistin showed the highest antibiofilm activity against the ESBL-producing *E. coli* strain *in vitro* and in the animal model. This combination presents a promising treatment option for implant-associated infections caused by fluoroquinolone-resistant Gram-negative bacilli and deserves further laboratory and clinical study.

ACKNOWLEDGMENTS

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